Applications of Non-Suppressed Ion Chromatography in Pharmaceutical and Clinical Analysis

Marguerite Y CROFT and Paul R HADDAD†
Department of Analytical Chemistry, University of New South Wales, PO Box 1, Kensington, NSW, 2033.

INTRODUCTION

Ion chromatography is a relatively new technique for rapid, sensitive, multianion analysis which has many advantages over alternative techniques such as wet chemical methods, spectrophotometry or the use of ion-selective electrodes. Since it was first described by Small et al[1] in 1975, ion chromatography has deservedly received a great deal of attention and is now routinely used in the analysis of environmental, water, food and industrial samples. However, applications on biological samples have been relatively few.

Ion chromatographic methods can be conveniently subdivided into suppressed and non-suppressed methods. The suppressed method utilises two ion-exchange columns: the first separates the solute anions, whilst the second column (generally referred to as the "suppressor" column) serves to reduce the background conductivity of the eluent in order to facilitate the use of conductivity detection. The most commonly used eluent in suppressed ion chromatography is carbonate/bicarbonate buffer and with this eluent, the suppressor column functions by protonating the eluent species to produce an essentially non-ionised weak acid which is poorly conducting. Most of the problems associated with this system arise from the suppressor column. A suppressed ion chromatograph is normally used as a dedicated instrument for ion analyses.

Non-suppressed ion chromatography was originally developed by Fritz et al[2] and involved the use of low capacity anion-exchange columns with low ionic strength eluents. This combination permitted direct conductivity detection (that is, without the requirement for a suppressor column) to be employed and was amenable to use on conventional liquid chromatographic instrumentation. Typical eluents used in non-suppressed ion chromatography are dilute solutions of aromatic acid salts such as phthalate or benzoate. Although originally designed for use with conductivity detection, this mode of ion chromatography is now commonly employed with a range of diverse monitoring techniques such as UV absorption, refractive index measurements or electrochemical detection.

In this paper, we investigate the possibility of applying non-suppressed ion chromatography to the determination of selected anions in biological and pharmaceutical samples. Two specific analyses are addressed: firstly the simultaneous determination of acetate, lactate, chloride and phosphate in intravenous solutions and secondly the determination of oxalate in urine. Acetate, lactate, chloride and phosphate are only weakly retained on anion-exchange columns and since previous attempts to simultaneously determine these species have not been successful, this analysis was considered to be an appropriate challenge for the separation capability of non-suppressed ion chromatography. The analysis of oxalate in urine was selected both for its clinical importance and as a representative analytical problem involving a complex sample matrix.

EXPERIMENTAL

Instrumentation

The liquid chromatograph used consisted of a Waters Associates (Milford, MA, USA) M6000 pump and U6K injector. Various detectors were used including a Waters Associates M450 variable wavelength detector, M430 conductivity detector and a homemade potentiometric flow-through detector incorporating a copper wire indicator electrode. The latter detector was connected to a Radiometer (Copenhagen, Denmark) pH M62 pH/millivolt meter and interfaced to a Houston Instruments (Austin, TS, USA) model 5271 10 mV chart recorder. Chromatograms obtained from the UV and conductivity detectors were recorded with a Waters Associates M730 data module.

Columns

The three anion-exchange columns used in this study were a Hamilton (Reno, NV, USA) PRP X-100

† Author to whom correspondence should be addressed.

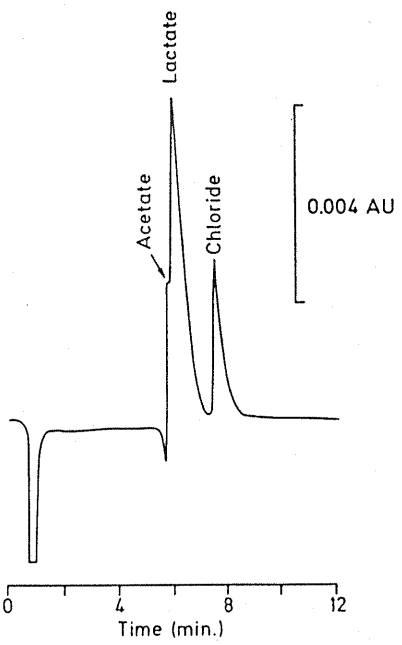

Figure 1. Separation of a mixture of acetate, lactate and chloride. Conditions. Column: Hamilton PRP X-100. Eluent: 0.1 mM potassium hydrogen phthalate (pH 6.0). Flow-rate: 2.0 ml/min. Injection volume: 10 μl. Indirect UV absorbance detection at 254 nm.

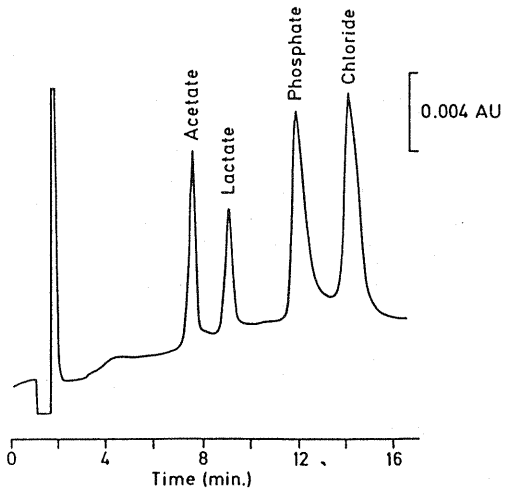

Figure 2. Separation of acetate, lactate, phosphate and chloride in a spiked IV solution. Conditions. Column: Hamilton PRP X-100. Eluent: 0.3 mM potassium hydrogen phthalate (pH 6.0) containing 30% acetonitrile. Flow-rate: 1.3 ml/min. Solute concentrations: 20-100 ppm. Indirect UV absorption detection at 285 nm.

ion chromatography column, 250 x 4.1 mm ID, a Waters Associates IC PAK A anion column, 50 x 4.6 mm ID, and a Vydac (The Separations Group, Hesperia, CA, USA) 300 IC anion chromatography column, 50 x 4.1 mm ID. A Waters Associates anion concentrator column, 6.0 x 5.0 mm ID was used as a guard column and was housed in the appropriate Guard-Pak precolumn module.

Procedures

Eluents were prepared using Analytical Grade reagents, chromatographic grade organic solvents and water purified on a Millipore (Bedford, MA, USA) Milli Q water purification system. All eluents were filtered through 0.45 μm membrane filters and degassed in an ultrasonic bath prior to use. The full strength gluconate/borate eluent (referred to as 100% gluconate eluent) contained 1.3 mM gluconate, 1.3 mM tetraborate and 5.8 mM boric acid.

Urine samples were freshly collected prior to analysis, diluted five times, ultrasonicated for 5 min and then passed through a Waters Associates C18 Sep-Pak cartridge which had previously been flushed with methanol. For studies involving acidified urine samples, 2 ml of either 1.0 M hydrochloric or nitric acid were added to a 2 ml aliquot of urine and the sample was allowed to stand for 30 min, after which time the sample was diluted to 10 ml and filtered through a 0.45 μm Millex filter.

RESULTS AND DISCUSSION

Separation of anions in intravenous solution

Acetate, lactate, chloride, phosphate, citrate and sulphate are found in intravenous solutions. The first four species are singly ionised (depending on pH) and are weakly retained on anion-exchangers, whereas the last two species are quite strongly retained and are easily separable, both from each other and from the singly ionised species. Analysis of intravenous solution is therefore limited by the requirement to separate the four weakly retained species. When a commercial low-capacity anion exchange column, such as the Hamilton PRP X-100, is applied to the separation of acetate, lactate, chloride and phosphate in an intravenous solution, poor resolution results, even when very dilute eluents are employed (Figure 1). It is clear that the selectivity of the eluent/column system is inadequate to achieve separation and that further selectivity effects must be explored in order to accomplish the desired separation.

The PRP X-100 column consists of quarternary ammonium functionalities bound to a styrenedivinylbenzene polymeric substrate. The low ion-exchange capacity of this column means that there are

appreciable areas of the column surface which are unfunctionalised and this leads to the column exhibiting a marked degree of reversed-phase character[3]. The column is also capable of withstanding high concentrations of organic modifier, so the addition of acetonitrile to the phthalate eluent was investigated as a potential method for improving the selectivity of the column towards the abovementioned solutes. It was found that a 30% acetonitrile solution containing 0.3 mM phthalate was an excellent eluent for the desired separation (Figure 2).

The improvement in resolution in the presence of acetonitrile was attributed to three factors. Firstly, the relative degrees of ionisation of the solute acids and the phthalate eluent were altered in comparison to the purely aqueous phthalate eluent, leading to the introduction of selectivity effects. Secondly, the mass transfer characteristics of the column were improved, leading to the production of sharper peaks. Thirdly, the retention mechanism appeared to be a combination of anion-exchange and reversed-phase behaviour, which further contributed to the ability of the column to differentiate between the solute species under study.

Calibration curves for all solutes were linear over the concentration range encountered in intravenous solutions and standard addition techniques were used to show that no matrix interference effects were present.

Determination of oxalate in urine

The determination of urinary oxalate levels is important in the diagnosis and treatment of certain pathological conditions, notably in patients with oxalate kidney stone disease and primary hyperoxaluria, which is an hereditary disorder. The requirement for oxalate assays in clinical studies has led to the development of a number of methods for the analysis of urinary oxalate. These include colorimetry[4], enzymatic techniques[5], isotope dilution analysis[6], isotachophoresis[7] and gas chromatography[8]. However, due to analytical difficulties resulting from the complexity of the sample matrix, many of these methods require extensive sample clean-up procedures or isolation of the oxalate from the sample prior to quantitation. These processes are generally time consuming and can lead to systematic errors in the analysis. Some liquid chromatographic methods have also been described and these include derivatisation followed by reversed-phase separation, ion-pair chromatography with UV absorption or amperometric detection, and suppressed ion chromatography with conductivity or electrochemical detection. In each of these procedures, interference effects or poor correlation with alternative techniques were observed.

The major problems encountered in the ion chromatographic analysis of oxalate in urine are the difficulty in resolving sulphate from oxalate and the fact that sulphate is present at a concentration approximately 100 times that of oxalate. Added to this is the regular practice of acidification of samples (usually with hydrochloric acid) in order to ensure dissolution of microcrystalline particles of calcium oxalate in the sample[9]. The presence of a large concentration of hydrochloric acid creates possible detection interferences, particularly in those detection methods which detect chloride ion.

A systematic study was undertaken of possible column, eluent and detector combinations suitable for the analysis of acidified and non-acidified samples. Three anion-exchange columns of different selectivities were examined, and direct UV absorption, indirect or "vacancy" UV absorption and conductivity were compared as detection modes. In short, none of the methods studied proved successful and all suffered either from serious interference effects from sulphate or severe baseline disturbance resulting from the acidity or chloride content of the treated samples. It was clear from these results that the ideal detection method required for this analysis should have high selectivity for oxalate in the presence of excess sulphate.

Such a detector has recently been developed in this laboratory[10,11] and is based on the measurement of the potential of a metallic copper wire electrode over which the column effluent passes. The copper electrode potential is governed by the concentration of copper ions present at the electrode surface, and this in turn depends on the natures of both the eluent and the solute under study. When a copper complexing solute such as oxalate is eluted, a change in electrode potential will occur due to the decrease in copper ion concentration resulting from complexation effects at the surface of the copper electrode. On the other hand, sulphate will not be detected under these conditions because it does not complex copper ions. The detector is therefore very selective towards oxalate in the presence of sulphate.

When the copper electrode detector was applied to the determination of oxalate, excellent response was observed and linear calibration plots were obtained over the concentration range 0-50 ppm of oxalate. Application of the method to the analysis of urine was also successful and a typical chromatogram is illustrated in Figure 3. Under the chromatographic conditions used in Figure 3, sulphate eluted at a retention time of 11.5 min, so it is evident that no interference from sulphate occurred. This observation was supported by the attainment of linear standard addition plots for oxalate additions to urine.

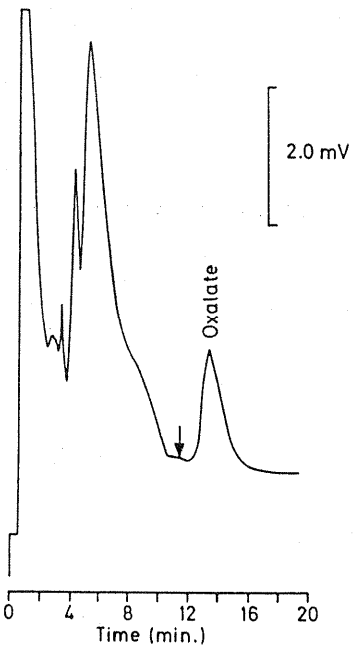

Figure 3. Potentiometric detection of oxalate in urine using a metallic copper indicator electrode. The arrow in the Figure marks the retention time of sulphate. Conditions. Column: Waters Associates IC PAK A. Eluent: 0.7 mM potassium hydrogen phthalate (pH 7.1). Injected volume: 10 μl of a 5 times diluted urine sample.

Urine samples acidified with hydrochloric acid did however present a problem in that the high chloride concentration destabilised the electrode response, presumably due to precipitation of cuprous chloride at the electrode surface. This problem could be partially circumvented by diverting the column effluent away from the potentiometric detector during the elution of chloride and then restoring flow through the detector prior to the elution of oxalate. However, a more suitable solution was to use nitric acid for sample acidification in preference to hydrochloric acid, since high concentrations of nitrate ion in the samples did not lead to the loss of electrode response.

CONCLUSIONS

This study has shown that non-suppressed ion chromatography can be successfully applied to the analysis of samples of biological importance, such as intravenous solutions and urine. It is probable that many further applications are also impossible, provided that sufficient attention is given to method development and sample clean-up procedures necessary to accommodate the complex nature of most samples encountered in this field.

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